

MATING AND EGG MASS PRODUCTION IN THE AEOLID
NUDIBRANCH *HERMISSENDA CRASSICORNIS*
(GASTROPODA: OPISTHOBRANCHIA)

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ABSTRACT

Interactions leading to copulation in the aeolid nudibranch, *Hermisenda crassicornis*, have a duration of only a minute or two, and intromission lasts only a few seconds (Longley and Longley, 1982). This paper reports additional details on the temporal structure and variation in structure of these interactions. It is also shown that sperm from a single copulation are sufficient to fertilize only 2 to 3 egg masses and that the state of an animal's sperm supply affects the rate at which it produces egg masses but not the size of the masses produced. In the discussion the results are compared to information on other simultaneous hermaphrodites in an effort to assess the possible adaptive advantages of the structure of the reproductive behavior of *H. crassicornis*.

INTRODUCTION

Longley and Longley (1982) reported recently that copulation in the nudibranch, *Hermisenda crassicornis*, is much briefer than that reported for many other opisthobranchs. In particular, copulation in this simultaneous hermaphrodite involves an explosive and reciprocal eversion of the penises with intromission lasting only a few seconds. In contrast, copulation in most other sea slugs lasts for many minutes or hours.

The gross sequential and temporal features of copulatory interactions in *H. crassicornis* are known, in part by default (Zack, 1975; Rutowski, 1982). Copulation is most likely to occur in interactions that begin when two animals meet head-to-head. After initial contact the participants stop forward movement and reciprocally touch each other with their tentacles. These repeated contacts were termed "flagellation" by Zack and last for about 45 s (Fig. 1A). The animals then begin moving slowly toward one another until they begin to pass, with the head of each animal moving along the right side of the other (Fig. 1B). Zack called this "sidling", and it brings the gonopores on the right side of the body a little behind the head into apposition. In Zack (1975) and Rutowski (1982) the cerata prevented observation of the eversion of the penises that occurs when the gonopores come into contact (Fig. 1C). Hence, interactions with sidling were not known to be copulatory until Longley and Longley's (1982) report. About 5 s after the beginning of sidling both animals erect their cerata and begin to move apart. This separation is often concurrent with lunging and biting by one or both animals directed at its partner (Fig. 1D). Within 20 s after erection of the cerata, the animals are fully separated.

The data presented in this report are directed at answering two questions about the intriguing mating behavior of *H. crassicornis*. First, what are the details of these

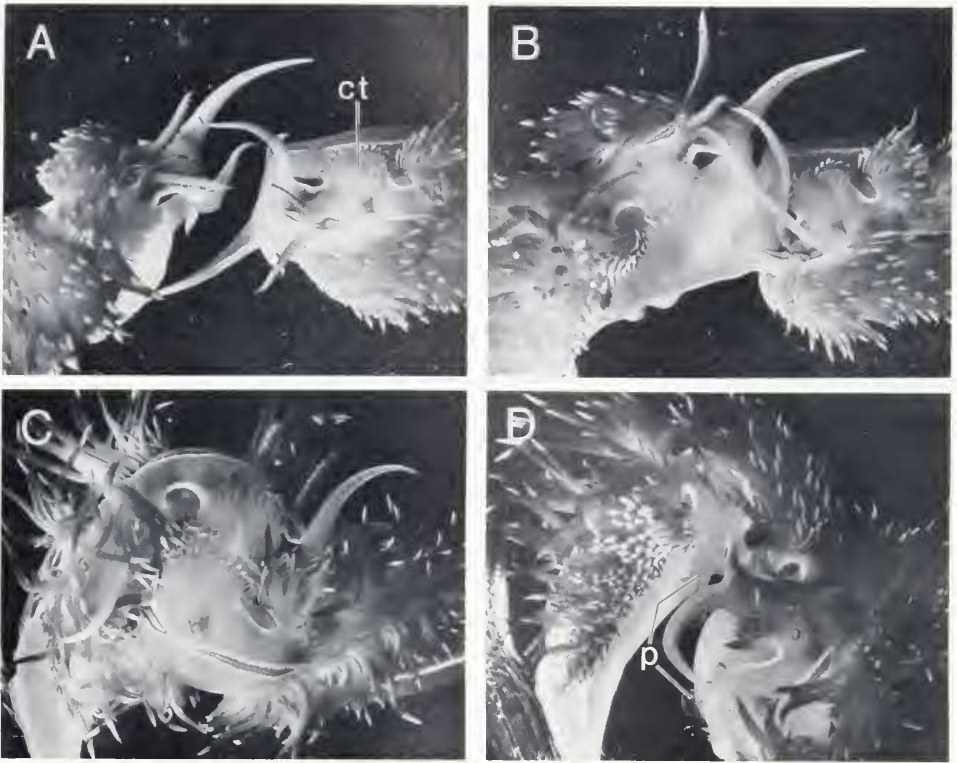


FIGURE 1. Events in a copulatory interaction in *Hermissenda crassicornis*. (A) Flagellation (*ct*, area above gonopore from which cerata have been cleared); (B) beginning of sidling; (C) the moment of intromission; (D) shortly after withdrawal (*p*, withdrawn but unretracted penis).

copulatory interactions? Second, how are egg fertility and output related to copulation? Special attention will be devoted to determining how long the sperm from a single copulation lasts relative to the duration of an individual's reproductive life. Quantitative and detailed answers to these questions are of interest because the Longleys' observations were largely qualitative and because the behavior of simultaneous hermaphrodites is generally so little known (Fischer, 1980). The discussion examines the adaptive features of the mating behavior of *H. crassicornis* relative to that of other opisthobranchs and other simultaneous hermaphrodites.

MATERIALS AND METHODS

Hermissenda crassicornis was collected between January and June in 1982 in the intertidal *Zostera marina* beds in Elkhorn Slough, Monterey County, CA. All animals were taken to the Long Marine Laboratory, Santa Cruz County, CA, where they were housed individually in small plastic cups (about 250 ml) each with its own supply of fresh running sea water (approximately 11–13°C) from a holding tank on the station property (Rutowski, 1982). The animals were fed either fresh mussel (*Mytilus californianus*) or, rarely, fresh squid mantle (*Loligo* spp.) every other day. At each feeding cups were cleared of food remaining from previous feedings and any detritus introduced by the sea water system.

The production and fertility of egg masses were carefully monitored for all isolated animals. If an animal produced an egg mass the animal was removed from its container and placed in a new one. The egg mass diameter was measured and then incubated undisturbed in unfiltered running sea water for 4 to 7 days at which time the shells of developing veligers become clearly visible (Williams, 1980). At this time the proportion of fertilized eggs in each mass was assessed by estimating, to the nearest 25%, the proportion of eggs that had developed into shelled veligers. In the results, any egg mass in which less than 50% of the eggs developed into veligers is referred to as an "infertile" egg mass. Those with 50% or more of the eggs developing into veligers are called "fertile" egg masses.

Isolated animals were, under conditions of constant observation, permitted to contact and mate with other individuals. The staging and videotaping of these interactions followed the techniques described in Rutowski (1982) except that larger containers (12 cm diameter watchglass or dish) were typically used.

Throughout the study the area above and slightly behind the gonopore of each animal was kept free of cerata (Fig. 1). This was done about once every week or two by plucking away with watchmakers forceps any cerata that might impede observations of penis eversion and intromission.

All parametric summary statistics are given as: mean \pm standard deviation. Statistical evaluations were made at the 0.05 level of significance.

RESULTS

Form of copulatory interactions

Over 60 interactions leading to sidling and copulatory attempts were observed during the course of this study. Of these, 43 were videotaped and analysed for the timing of events before and after penis eversion (Table I, Fig. 1).

The form of all these interactions was similar to the copulatory interactions described by Longley and Longley (1982) and for interactions with flagellation and sidling described by Zack (1975) and Rutowski (1982). I would add that during flagellation there is a pronounced swelling of the gonopore region that continues until penis eversion. If flagellation is terminated for some reason the swelling decreases and a small amount of semen is released from the gonopore. Also, new temporal information to add to these descriptions includes the time from the beginning of sidling (1) to penis eversion and (2) until both animals have uncoupled (Table I). These data confirm the relatively rapid progression of events in these matings. The average duration of intromission was only about 4 s, and in all but a few cases differences in the time of eversion between participants were not resolvable with the video system used.

TABLE I

The timing of events in a copulatory interaction from the beginning of sidling until the animals begin to retreat or move apart

Event	Time of occurrence relative to beginning of sidling (s)	Source
Cerata movement	4.11 \pm 0.859 s (n = 19)	Rutowski, 1982
Intromission	6.53 \pm 2.77 s (n = 39)	This study
Withdrawal	10.5 \pm 2.88 s (n = 31)	This study
Begin retreat	13.1 \pm 6.2 s (n = 19)	Rutowski, 1982

Sidling does not always lead to intromission. First, on a few rare occasions one or both animals did not evert the penis. When neither animal attempted intromission, they simply moved apart after a brief apposition of the gonopores. Second, as pointed out by the Longleys, not all penis eversion resulted in intromission. Typically, during the process of eversion in such an interaction, the penis appeared to ricochet off the body wall of the partner next to its gonopore and ejaculated the semen at the moment of full extension. Of the 41 videotape records of interactions with sidling and penis eversion, 37 were of adequate quality to determine whether or not penis eversion with intromission occurred. In 38% of these both animals attained intromission. However, in another 49%, although both animals tried, one animal missed the other's gonopore. In the remaining interactions neither animal attained intromission either because both missed (8%) or because one missed and one did not attempt intromission (5%). Interactions in which neither animal attempted penis eversion were not included in this count. In summary, sperm transfer was not reciprocal in almost 50% of the interactions in which penis eversion by both animals occurred.

After penis eversion and separation a new behavior pattern was observed which was directed at the mass of semen that was frequently seen in the vicinity of the gonopore particularly if intromission did not occur. An animal stopped, turned its head so that the mouth was positioned near the gonopore, and consumed all or part of the semen in the vicinity of the gonopore (Fig. 2). Data on the frequency of occurrence of this behavior was not recorded. It also occurs in land snails in the genus *Partula* (Lipton and Murray, 1979).

Effect of copulation on egg production

Rates of egg production: fertile versus infertile. If an individual of *H. crassicornis* is isolated and denied contact with conspecifics it will typically produce a few com-



FIGURE 2. *Hermisenda crassicornis* immediately after copulation ingesting semen from the vicinity of the gonopore.

pletely (100%) fertile egg masses and then either stop producing eggs or begin to produce masses with an increasing proportion of infertile eggs (Table II). In egg masses with fertile and infertile eggs, the infertile eggs are concentrated in the outer loops of the spiral while those eggs near the middle of the spiral are mostly fertile. The average interval between fertile egg masses (as defined earlier) was 4.32 ± 2.22 days (Fig. 3). The time between the last fertile mass produced by an animal and the first infertile mass was 9.35 ± 10.06 days, which is significantly different from the interval between fertile masses (Wilcoxon rank sum test, $P = 0.00007$; $t = 5.47$, $P < 0.001$). The average interval between masses of mostly infertile eggs was 7.36 ± 6.5 days which was also significantly longer than the time between two fertile masses (Wilcoxon rank sum test, $P = 0.00005$; $t = 5.19$, $P < 0.0001$) but not significantly different from the time between the last fertile and the first infertile egg mass (Wilcoxon rank sum test, $P = 0.17$; $t = 1.17$, $P > 0.2$).

Another way of summarizing these data is to ask, if an animal produces a fertile egg mass how does the probability that the next mass will be infertile change with the time elapsed between the two? As Table III shows, if the interval is greater than 11 days the probability that the next mass is infertile is 100%. Hence for purposes of this study an animal was regarded as sperm depleted if (1) it had produced 2 or more infertile egg masses, or (2) if it had not produced a fertile egg mass in 20 days or more.

Egg mass size: fertile versus infertile. Egg mass size is known to be a function of the size of the producer in *H. crassicornis* (Harrigan and Alkon, 1978). To minimize this effect I examined the diameter of egg mass pairs that were sequentially produced by the same animal, although no more than 11 days apart, and that varied by at least 50 percent in the proportion of eggs fertilized. Of 28 pairs of egg masses produced the more fertile mass was the larger of the two in 14 while the opposite situation appeared in 13 pairs. In one pair there was no difference. I conclude that the state of an animal's sperm supply has no consistent effect on the size of the egg mass that it produces.

Effect of copulation on the rate of fertile egg mass production. Thirty-eight animals that were sperm depleted (by the criteria above) were each permitted to engage in one sidling interaction with another animal and then returned to isolation. As indicated by the videotape records, 28 of these animals were successfully intromitted, while 15 were not. For 93% of those that were successfully intromitted, the next egg mass they produced was fertile. All produced egg masses an average of 2.64 ± 1.33 days after the copulation. These included 11 animals that had not produced an egg mass in over 20 days. In contrast, of the 15 animals that were not inseminated, none produced fertile egg masses within 20 days of the interaction with sidling. In

TABLE II

A summary of the fertility of egg masses produced after the last completely (100%) fertile egg mass produced

Egg mass after last 100% fertile mass	Percent of egg masses in which the % of eggs that developed was:				Sample size
	0-24	25-49	50-74	75-100	
First	11%	48%	15%	26%	27
Second	63%	31%	6%	0%	16
Third	100%	0%	0%	0%	11
Fourth and beyond	100%	0%	0%	0%	6

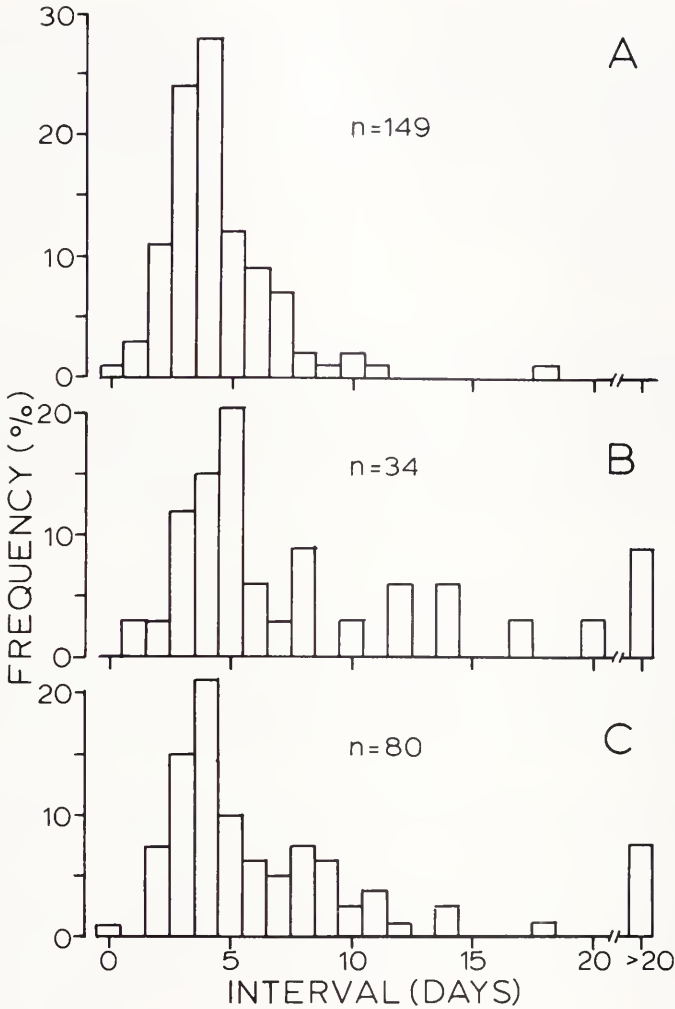


FIGURE 3. Intervals between successive egg masses. (A) Fertile to fertile; (B) fertile to infertile; (C) infertile to infertile.

fact, 5 of these animals did not produce any egg mass in the first 20 days after the interaction.

The likelihood that an isolated animal will produce an infertile egg mass increases with the number of egg masses produced since the last successful copulation (Table IV). By the fifth egg mass after copulation the probability that the mass is infertile is 50% or greater. The average number of fertile masses produced by an isolated animal after a single copulation and before it shows clear signs of sperm depletion is 2.65 ± 1.66 masses ($n = 20$).

Egg production of isolated wild-caught individuals

Ten animals were isolated from the time of capture in the field and their subsequent egg production was monitored in the laboratory for a period of 24 days.

TABLE III

The relationship between the fertility of an egg mass and the time of its production relative to the last fertile egg mass (no intervening masses)

Interval (days)	% Infertile	Sample Size
0-2	12.5	16
3-5	18.2	99
6-8	23.3	30
9-11	33.3	6
12 or more	100	10

During this time all produced at least one fertile egg mass; but before the end, 7 showed signs of sperm depletion. Two of these 7 produced infertile egg masses while the other five did not produce any egg masses during the final 10 days or more of isolation. The average number of fertile egg masses produced during the 24 day period was 3.7 ± 2.5 . This information suggests that about 80% of these animals carried stored sperm from a recent copulation or recent copulations.

DISCUSSION

Comparison with reports on copulation in other opisthobranchs

These data confirm Longley and Longley's (1982) report that copulation in *H. crassicornis* is a rapid affair. Copulatory interactions last a few minutes, but the actual duration of intromission is only a few seconds. It appears that one consequence of such a rapid attempt at intromission is the high frequency of unsuccessful attempts.

The Longleys point out that the hesitant approach and high willingness to turn away from contact that characterize the behavior of participants in the early phases of all interactions in *H. crassicornis* may be an effort on the part of one or both animals to avoid cannibalism, which has been observed in several studies of this animal (Zack, 1975; Rutowski, 1982). It may also be that the speed of copulation also reflects an adaptation that minimizes the duration of contact with potentially cannibalistic conspecifics. Supporting this notion is the observation that cannibalism and apparently cannibalistic attacks on conspecifics have not been reported for species of nudibranchs in which intromission is known to last many minutes or hours, such as *Embletonia fuscata* (Chambers, 1934), *Coryphella stimpsoni* (Morse, 1971), *Precuthona peachii* (Christensen, 1977), *Tritonia hombergi* (Thompson,

TABLE IV

A summary of the fertility of egg masses produced by initially sperm depleted animals that were intromitted

Egg mass after copulation	% Infertile	Sample Size
First	7.1	28
Second	18.5	27
Third	38.5	26
Fourth	65	20
Fifth	50	10
Sixth	57	7
Seventh and Eighth	80	5

1961), and *Chromodoris zebra* (Crozier, 1918). Longley and Longley (1982) report a very brief copulation in *Aeolidia papillosa* in which I have observed cannibalistic attacks on conspecifics. However, the tectibranch, *Navanax inermis*, is also known to feed readily on conspecifics but its copulation is prolonged (Paine, 1965).

The continued production of egg masses in spite of sperm depletion is known for a number of nudibranchs (Hadfield, 1963; Harris, 1975; Rivest, 1978; Christensen, 1977) and is puzzling. Why an animal continues even at a reduced rate to invest energy and resources in egg production when most will not develop is not currently clear.

Comparisons with other simultaneous hermaphrodites

In the opisthobranchs, reciprocal and internal fertilization is the rule (Costello, 1938; Beeman, 1977). In most, reciprocal insemination is simultaneous. Only in *Aplysia* has the assumption of single sex roles by individual animals been reported (Lederhendler and Tobach, 1977). In *Hypoplectrus nigricans*, a simultaneously hermaphroditic serranid fish, fertilization is external but reciprocal in that the animals take turns playing male and female roles in mating interactions (Fischer, 1980). The prevalence of reciprocity in these simultaneous hermaphrodites is in some ways unexpected. The best interests of the male and female functions of a hermaphrodite's reproductive tract are not the same. Charnov (1979) points out that in simultaneous hermaphrodites copulation is probably primarily an effort to give sperm rather than receive it. In other words, the fitness through male function can best be maximized through repeated copulation. It follows then that in mating interactions simultaneous hermaphrodites should be more often willing to play a male than a female role. One might expect to see more one-way inseminations or efforts on the part of individuals to give sperm without receiving it. However, at any given time in a population there will be many more animals willing to give sperm than to receive it so that to maximize the rate of giving sperm an animal must reduce its reluctance to receive it. As Charnov states, "each should be inclined to accept sperm in order to give its sperm away." In this respect the mating behavior of simultaneous hermaphrodites is more like that expected in cooperative interactions than those of gonochoric organisms. This view has been promulgated by Axelrod and Hamilton (1981) in their analysis of the selection pressures shaping the form of cooperative interactions in animals.

There are two ways in which this analysis might be relevant to understanding the reproductive behavior of *H. crassicornis*. First, it is possible that the missed intromissions represent efforts by animals to give sperm without receiving it by actively deflecting the penis of the other individual. Second, it is at least plausible that the rapidity of intromission is a result of animals attempting to give sperm quickly without giving the other animal a chance to intromit. These ideas both seem unlikely, however, in that most of the missed individuals in this study were animals in a sperm-depleted state who could have greatly benefited from receiving as well as giving sperm.

Evolution of simultaneous hermaphroditism in H. crassicornis

The data presented here and elsewhere permit a partial evaluation of the applicability of competing hypotheses for the adaptive significance of simultaneous hermaphroditism in *H. crassicornis*. Currently, there are three major explanations of this sort. The first two have been summarized by Ghiselin (1969). First, simultaneous hermaphroditism may increase an animal's reproductive efficiency by per-

mitting self-fertilization. This idea does not apply to *H. crassicornis* because this animal has never been observed to self-fertilize (Harrigan and Alkon, 1978; this study). The second explanation is the low density model which suggests that simultaneous hermaphroditism will be favored in organisms with low population densities in which contacts with conspecifics are rare or infrequent. Any conspecific is then an appropriate mate. Although this model is often invoked to explain the occurrence of simultaneous hermaphroditism in nudibranchs (e.g., Todd, 1978) it does not appear to fit *H. crassicornis* well. If contact with conspecifics are rare, one might expect that (1) sperm from a single copulation would last the better part of an individual's active reproductive life and (2) individuals should readily mate in any contact with a conspecific. Neither of these conditions is met in *H. crassicornis*. Individuals of *H. crassicornis* live several months in the laboratory (Harrigan and Alkon, 1978; pers. obs.) and presumably in the field as well. The sperm from a single copulation is sufficient to fertilize most eggs in about 3 egg masses which if produced once every 4 to 5 days will last an animal about 15 days or less. This coupled with the surprisingly high frequency of unsuccessful copulation attempts suggests that these animals must mate several times during their life to maintain a maximal egg production rate. The lack of precision in intromission and in the production of fertile eggs is also not to be expected if the low density model were in force. Furthermore, as Zack (1975) points out, most contacts and interactions in *H. crassicornis* do not end in the sidling that often leads to copulation. If anything, the animals appear more inclined to cannibalize than to copulate with conspecifics. In sum, the behavior of these animals does not conform to some simple expectations about behavior derived from the low density model.

The third explanation is a form of Maynard Smith's (1978) resource allocation model, which states that the fitness return per egg will diminish as the number of eggs produced increases, especially in species that produce myriads of small propagules. Hence, over evolutionary time selection might favor a partitioning of reproductive energies into both male and female modes. The only observation supporting the application of this model to *H. crassicornis* is that they do appear readily able to produce millions of eggs (Williams, 1980) and so simultaneous investment in male functions might well payoff. Of the three adaptationist models, this one, largely by elimination, appears to be the most likely explanation for the evolution of simultaneous hermaphroditism in this nudibranch.

In closing, it cannot be discounted at this time that the occurrence of simultaneous hermaphroditism may be a result of phylogenetic inertia. In other words, this reproductive mode need not be adaptive in *H. crassicornis* but may have retained during evolution as an incidental effect of other advantageous life history traits. This view is supported by the prevalence of this reproductive mode in other opisthobranchs which suggests that the ancestors of *H. crassicornis* were simultaneous hermaphrodites. Hence, this model and the resource allocation model appear to be the major competing hypotheses for the explanation of simultaneous hermaphroditism in *H. crassicornis*.

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